PATENT
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Tracey Simmons
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Tracey Simmons
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Philip E. Branton et al.	Art Unit:	1633
Serial No.:	09/214,478	Examiner:	S. Chen
Filed:	June 7, 1999		
Title:	ADENOVIRUS E4 PROTEINS FOR INDUCING CELL DEATH		

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF DR. PHILIP BRANTON

1. I am a joint inventor on the above-referenced patent application.

2. Any description in Marcellus et al., J. Virol. 70:6207-6215, 1996 (hereafter "Marcellus") was the joint contribution of Philip Branton, Richard Marcellus, Jose Teodoro, and Gordon Shore, each of whom is an inventor in the above-captioned case, notwithstanding the inclusion of the additional authors, Tim Wu, Douglas Brough, and Gary Ketner, who contributed to other work described in this paper.

3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further

that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: July 16, 2001

Philip E. Branton, Ph.D.

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Tracey Simmons — Tracey Simmons
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Assistant Commissioner For Patents
Washington, D.C. 20231

DECLARATION OF DR. PHILIP BRANTON UNDER 37 C.F.R. § 1.132
TRAVERSING GROUNDS OF REJECTION

Under 37 C.F.R. § 1.132 and regarding the rejection of the claims for lack of enablement, I declare:

1. I am an inventor of the subject matter described and claimed in the above-referenced patent application.

2. To demonstrate that a vector encoding an E4orf4 polypeptide was capable of increasing apoptosis *in vivo*, individuals acting under my direction

performed the following experiment. H1299 human lung carcinoma cells or C33A human cervical carcinoma cells ($1X10^7$ cells) were implanted subcutaneously into nude mice. Once initial tumors were produced, they were surgically removed, cut into small pieces, and the reimplanted subcutaneously into fresh six week-old nude mice to produce clonal tumors. Once the tumors reached approximately 5 mm in diameter, treatment with a tetracycline-inducible E4orf4-expressing adenovirus was commenced. Mice were provided with water containing 2 mg/ml doxycycline to activate promoter expression. The adenovector was purified and diluted to a titer of $1X10^{10}$ pfu/ml. Virus (100 µl or $1X10^9$ pfu) or control was injected directly into the tumor daily for five days (with C33A xenografts) or 10 days (with H1299 xenografts). Tumors were measured daily for one month and the tumor volumes were calculated and plotted.

3. The data from the foregoing experiments are depicted in Exhibits A and B. When treated with an E4orf4-encoding adenovector, xenografted H1299 tumors had a tumor volume that was 10% that of PBS-treated controls (Exhibit A). A similar finding was observed with xenografted C33A tumors; tumor volume was reduced by more than half (Exhibit B). Based on these *in vivo* data, and on *in vitro* data, described in the specification,

that demonstrate E4orf4 expression in mammalian tumor cells induces apoptosis, I conclude that E4orf4 induces apoptosis in the xenografted tumor cells.

4. The xenograft model is an excellent and well-accepted model for the study of human neoplasms and therapies. It is routine for therapies which show promise for the treatment of tumors in this *in vivo* model to proceed to human trials.

5. The experiments performed in the present patent application were performed using E4orf4 nucleic acids and polypeptides derived from adenoviral serotype Ad2. As is demonstrated in the specification, Applicants postulated that it was highly likely that E4orf4 nucleic acids and polypeptides from other adenoviral serotypes would, like their Ad2 counterparts, induce apoptosis. Moreover, Applicants taught that the identification of E4orf4 in other adenoviral serotypes could readily be achieved using standard techniques.

6. Individuals acting under my direction ordered adenoviruses of serotype Ad3, Ad4, Ad9, Ad12, and Ad40 from the American Type

Culture Collection (Manassas, VA). The viruses were used to infect human cells in culture, and nucleic acids encoding E4orf4 were amplified using the polymerase chain reaction under standard conditions. As is demonstrated in table, the E4orf4 nucleic acid sequences were 44-53% identical to that of Ad2 E4orf4.

Table

Strain	% Identity to Ad2
Ad3	44
Ad9	53
Ad12	51
Ad40	48

7. Using the cell killing assay described in the specification, all but one of the E4orf4 genes were capable of inducing apoptosis (Exhibit C). The only E4orf4 polypeptide that did not induce apoptosis, Ad3, was found to be expressed at lower levels than the others under the conditions tested, indicating that it may be insufficient expression levels, and not the polypeptide itself, that is the cause of the failure of the polypeptide to induce apoptosis.

8. Individuals acting under my direction performed a systematic substitution of single or multiple amino acid residues of Ad2

serotype E4orf4. Amino acid substitution is performed using standard techniques. We found that the following changes do not alter E4orf4 biological activity: P10A, C18A, Y26A, D31A/V32A/R34A, H38A, Y42A, E44A, P45A, E46A/R48A, R48A, Y59A, C78A, C85A, D99A, and S106A (the first letter is the wild-type amino acid from Ad2 serotype, the number is the residue, and the last letter is the substituted amino acid).

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

July 16, 2001
Date

Philip Branton, Ph.D.

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